



PCT10

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RAW SEQUENCE LISTING  
 PATENT APPLICATION: US/10/069,040

DATE: 03/07/2002  
 TIME: 15:35:09

Input Set : A:\pto.vsk.txt  
 Output Set: N:\CRF3\03072002\J069040.raw

3 <110> APPLICANT: Novozymes A/S  
 4 Svendsen, Allan  
 5 Andersen, Carsten  
 6 Borchert, Torben Vedel  
 8 <120> TITLE OF INVENTION: Pullulanase variants and methods for preparing such variants  
 with  
 9 predetermined properties  
 11 <130> FILE REFERENCE: 6072.204-US  
 C--> 13 <140> CURRENT APPLICATION NUMBER: US/10/069,040  
 C--> 14 <141> CURRENT FILING DATE: 2002-02-19  
 16 <160> NUMBER OF SEQ ID NOS: 40  
 18 <170> SOFTWARE: PatentIn version 3.1  
 20 <210> SEQ ID NO: 1  
 21 <211> LENGTH: 2766  
 22 <212> TYPE: DNA  
 23 <213> ORGANISM: Bacillus acidopullulyticus  
 25 <220> FEATURE:  
 26 <221> NAME/KEY: CDS  
 27 <222> LOCATION: (1)..(2766)  
 28 <223> OTHER INFORMATION:  
 31 <400> SEQUENCE: 1  
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 33 Asp Ser Thr Ser Thr Lys Val Ile Val His Tyr His Arg Phe Asp Ser  
 34 1 5 10 15  
 36 aac tat acg aat tgg gac gtc tgg atg tgg cct tat cag cct gtt aat 96  
 37 Asn Tyr Thr Asn Trp Asp Val Trp Met Trp Pro Tyr Gln Pro Val Asn  
 38 20 25 30  
 40 ggt aat gga gca gct tac caa ttc act ggt aca aat gat gat ttt ggc 144  
 41 Gly Asn Gly Ala Ala Tyr Gln Phe Thr Gly Thr Asn Asp Asp Phe Gly  
 42 35 40 45  
 44 gct gtt gca gat acg caa gtg cct gga gat aat aca caa gtt ggt ttg 192  
 45 Ala Val Ala Asp Thr Gln Val Pro Gly Asp Asn Thr Gln Val Gly Leu  
 46 50 55 60  
 48 att gtt cgt aaa aat gat tgg agc gag aaa aat aca cca aac gat ctc 240  
 49 Ile Val Arg Lys Asn Asp Trp Ser Glu Lys Asn Thr Pro Asn Asp Leu  
 50 65 70 75 80  
 52 cat att gac ctt gca aaa ggc cat gaa gta tgg att gta caa ggg gat 288  
 53 His Ile Asp Leu Ala Lys Gly His Glu Val Trp Ile Val Gln Gly Asp  
 54 85 90 95  
 56 cca act att tat tac aat ctg agc gac gca cag gct gcc gca ata cca 336  
 57 Pro Thr Ile Tyr Tyr Asn Leu Ser Asp Ala Gln Ala Ala Ile Pro  
 58 100 105 110  
 60 tct gtt tca aat gcc tat ctt gat gat gaa aaa aca gta cta gca aag 384  
 61 Ser Val Ser Asn Ala Tyr Leu Asp Asp Glu Lys Thr Val Leu Ala Lys

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62	115	120	125	
64	cta agt atg ccg atg acg ctg gcg gat gct gca agc ggc ttt acg gtt			432
65	Leu Ser Met Pro Met Thr Leu Ala Asp Ala Ala Ser Gly Phe Thr Val			
66	130	135	140	
68	ata gat aaa acc aca ggt gaa aaa atc cct gtc acc tct gct gta tcc			480
69	Ile Asp Lys Thr Thr Gly Glu Lys Ile Pro Val Thr Ser Ala Val Ser			
70	145	150	155	160
72	gca aat ccg gta act gcc gtt ctt gtt gga gat tta caa cag gct ttg			528
73	Ala Asn Pro Val Thr Ala Val Leu Val Gly Asp Leu Gln Gln Ala Leu			
74	165	170	175	
76	gga gca gcg aat aat tgg tca cca gat gat gat cac aca ctg cta aaa			576
77	Gly Ala Ala Asn Asn Trp Ser Pro Asp Asp Asp His Thr Leu Leu Lys			
78	180	185	190	
80	aag ata aat cca aac ctt tac caa tta tcg ggg aca ctt cca gct ggt			624
81	Lys Ile Asn Pro Asn Leu Tyr Gln Leu Ser Gly Thr Leu Pro Ala Gly			
82	195	200	205	
84	aca tac caa tat aag ata gcc ttg gac cat tct tgg aat acc tcc tat			672
85	Thr Tyr Gln Tyr Lys Ile Ala Leu Asp His Ser Trp Asn Thr Ser Tyr			
86	210	215	220	
88	cca ggt aac aat gta agt ctt act gtt cct cag gga ggg gaa aag gtt			720
89	Pro Gly Asn Asn Val Ser Leu Thr Val Pro Gln Gly Gly Glu Lys Val			
90	225	230	235	240
92	acc ttt acc tat att cca tct acc aac cag gta ttc gat agc gtc aat			768
93	Thr Phe Thr Tyr Ile Pro Ser Thr Asn Gln Val Phe Asp Ser Val Asn			
94	245	250	255	
96	cat cct aac caa gca ttc cct aca tcc tca gca ggg gtc cag aca aat			816
97	His Pro Asn Gln Ala Phe Pro Thr Ser Ser Ala Gly Val Gln Thr Asn			
98	260	265	270	
100	tta gtc caa ttg act tta gcg agt gca ccg gat gtc acc cat aat tta			864
101	Leu Val Gln Leu Thr Leu Ala Ser Ala Pro Asp Val Thr His Asn Leu			
102	275	280	285	
104	gat gta gca gca gac ggt tac aaa gcg cac aat att tta cca agg aat			912
105	Asp Val Ala Ala Asp Gly Tyr Lys Ala His Asn Ile Leu Pro Arg Asn			
106	290	295	300	
108	gtt tta aat ctg ccg cgg tat gat tat agt gga aat gat ttg ggt aat			960
109	Val Leu Asn Leu Pro Arg Tyr Asp Tyr Ser Gly Asn Asp Leu Gly Asn			
110	305	310	315	320
112	gtt tat tca aag gat gca aca tcc ttc cgg gta tgg gct cca aca gct			1008
113	Val Tyr Ser Lys Asp Ala Thr Ser Phe Arg Val Trp Ala Pro Thr Ala			
114	325	330	335	
116	tcg aat gtc cag ttg ctt tta tac aat agt gag aaa ggt tca ata act			1056
117	Ser Asn Val Gln Leu Leu Tyr Asn Ser Glu Lys Gly Ser Ile Thr			
118	340	345	350	
120	aaa cag ctt gaa atg caa aag agt gat aac ggt aca tgg aaa ctt cag			1104
121	Lys Gln Leu Glu Met Gln Lys Ser Asp Asn Gly Thr Trp Lys Leu Gln			
122	355	360	365	
124	gtt tct ggt aat ctt gaa aac tgg tat tat cta tat caa gtc aca gtg			1152
125	Val Ser Gly Asn Leu Glu Asn Trp Tyr Tyr Leu Tyr Gln Val Thr Val			
126	370	375	380	

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128 aat ggg aca aca caa acg gca gtt gat cca tat gcg cgt gct att tct	1200
129 Asn Gly Thr Thr Gln Thr Ala Val Asp Pro Tyr Ala Arg Ala Ile Ser	
130 385 390 395 400	
132 gtc aat gca aca cgc ggt atg att gtg gac cta aaa gct acc gat cct	1248
133 Val Asn Ala Thr Arg Gly Met Ile Val Asp Leu Lys Ala Thr Asp Pro	
134 405 410 415	
136 gca ggg tgg cag gga gat cat gaa cag aca cct gcg aat cca gta gat	1296
137 Ala Gly Trp Gln Gly Asp His Glu Gln Thr Pro Ala Asn Pro Val Asp	
138 420 425 430	
140 gaa gtg att tat gaa gcg cat gta cgc gat ttt tcg att gat gct aat	1344
141 Glu Val Ile Tyr Glu Ala His Val Arg Asp Phe Ser Ile Asp Ala Asn	
142 435 440 445	
144 tca ggt atg aaa aat aaa ggg aag tat tta gcg ttt aca gag cat gga	1392
145 Ser Gly Met Lys Asn Lys Gly Lys Tyr Leu Ala Phe Thr Glu His Gly	
146 450 455 460	
148 aca aaa gga ccg gat cat gta aag aca ggt att gat agt ttg aag gaa	1440
149 Thr Lys Gly Pro Asp His Val Lys Thr Gly Ile Asp Ser Leu Lys Glu	
150 465 470 475 480	
152 ttg ggc atc acc act gtt caa ttg caa cct gtt gag gag ttt aac agt	1488
153 Leu Gly Ile Thr Thr Val Gln Leu Gln Pro Val Glu Glu Phe Asn Ser	
154 485 490 495	
156 att gat gag acc cag cct gat acg tat aac tgg ggc tac gat cca agg	1536
157 Ile Asp Glu Thr Gln Pro Asp Thr Tyr Asn Trp Gly Tyr Asp Pro Arg	
158 500 505 510	
160 aac tat aac gta cca gag gga gct tat gcc aca act cca gaa gga aca	1584
161 Asn Tyr Asn Val Pro Glu Gly Ala Tyr Ala Thr Thr Pro Glu Gly Thr	
162 515 520 525	
164 gcg cgt ata aca gaa tta aag caa tta att caa agc ctt cat cag cag	1632
165 Ala Arg Ile Thr Glu Leu Lys Gln Leu Ile Gln Ser Leu His Gln Gln	
166 530 535 540	
168 cgg att ggt gtc aat atg gat gtt gtt tat aac cat acc ttt gat gtg	1680
169 Arg Ile Gly Val Asn Met Asp Val Val Tyr Asn His Thr Phe Asp Val	
170 545 550 555 560	
172 atg gtt tct gat ttt gat aaa att gtc ccg caa tat tat tat cgt acc	1728
173 Met Val Ser Asp Phe Asp Lys Ile Val Pro Gln Tyr Tyr Tyr Arg Thr	
174 565 570 575	
176 gat agt aat ggc aat tat acg aac gga tca ggt tgc ggc aat gaa ttc	1776
177 Asp Ser Asn Gly Asn Tyr Thr Asn Gly Ser Gly Cys Gly Asn Glu Phe	
178 580 585 590	
180 gcg act gag cat cca atg gca caa aag ttt gtg ctt gat tca gtt aat	1824
181 Ala Thr Glu His Pro Met Ala Gln Lys Phe Val Leu Asp Ser Val Asn	
182 595 600 605	
184 tat tgg gta aat gag tac cac gtg gat ggc ttc cgt ttt gac tta atg	1872
185 Tyr Trp Val Asn Glu Tyr His Val Asp Gly Phe Arg Phe Asp Leu Met	
186 610 615 620	
188 gct ctt tta gga aaa gac acg atg gca aaa ata tca aac gag ctg cat	1920
189 Ala Leu Leu Gly Lys Asp Thr Met Ala Lys Ile Ser Asn Glu Leu His	
190 625 630 635 640	
192 gcc att aat cct ggt att gtt tta tat gga gaa cca tgg act ggc ggc	1968

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193 Ala Ile Asn Pro Gly Ile Val Leu Tyr Gly Glu Pro Trp Thr Gly Gly		
194 645 650 655		
196 aca tcg gga tta tct agc gac cag ctt gta acg aag ggt caa caa aag		2016
197 Thr Ser Gly Leu Ser Ser Asp Gln Leu Val Thr Lys Gly Gln Gln Lys		
198 660 665 670		
200 gga tta gga att ggc gtt ttc aac gat aat ata cgt aat ggg ctc gat		2064
201 Gly Leu Gly Ile Gly Val Phe Asn Asp Asn Ile Arg Asn Gly Leu Asp		
202 675 680 685		
204 ggg aac gtg ttt gat aaa acg gca caa ggc ttt gca aca gga gat cca		2112
205 Gly Asn Val Phe Asp Lys Thr Ala Gln Gly Phe Ala Thr Gly Asp Pro		
206 690 695 700		
208 aac cag gtg gat gtc att aaa aat gga gta atc ggt agt att caa gat		2160
209 Asn Gln Val Asp Val Ile Lys Asn Gly Val Ile Gly Ser Ile Gln Asp		
210 705 710 715 720		
212 ttt act tca gca cct agc gaa acg att aac tat gtt aca agc cat gat		2208
213 Phe Thr Ser Ala Pro Ser Glu Thr Ile Asn Tyr Val Thr Ser His Asp		
214 725 730 735		
216 aac atg acg ctt tgg gat aaa att tta gca agt aat cca agt gac act		2256
217 Asn Met Thr Leu Trp Asp Lys Ile Leu Ala Ser Asn Pro Ser Asp Thr		
218 740 745 750		
220 gag gct gac cga att aaa atg gat gaa ttg gca cat gcc gta gta ttc		2304
221 Glu Ala Asp Arg Ile Lys Met Asp Glu Leu Ala His Ala Val Val Phe		
222 755 760 765		
224 act tca caa ggt gta cca ttt atg caa ggt gga gaa gaa atg ctg agg		2352
225 Thr Ser Gln Gly Val Pro Phe Met Gln Gly Gly Glu Glu Met Leu Arg		
226 770 775 780		
228 aca aaa ggc gga aat gat aac agt tat aac gct gga gat agt gtg aat		2400
229 Thr Lys Gly Gly Asn Asp Asn Ser Tyr Asn Ala Gly Asp Ser Val Asn		
230 785 790 795 800		
232 cag ttc gac tgg tca aga aag gcg caa ttt aag gat gtt ttt gac tac		2448
233 Gln Phe Asp Trp Ser Arg Lys Ala Gln Phe Lys Asp Val Phe Asp Tyr		
234 805 810 815		
236 ttt tct agt atg att cat ctt cgt aat cag cac ccg gca ttc agg atg		2496
237 Phe Ser Ser Met Ile His Leu Arg Asn Gln His Pro Ala Phe Arg Met		
238 820 825 830		
240 acg aca gcg gat caa att aaa cag aat ctt aca ttc tta gaa agc cca		2544
241 Thr Thr Ala Asp Gln Ile Lys Gln Asn Leu Thr Phe Leu Glu Ser Pro		
242 835 840 845		
244 aca aac acg gta gct ttc gag tta aag aat tat gca aac cat gat aca		2592
245 Thr Asn Thr Val Ala Phe Glu Leu Lys Asn Tyr Ala Asn His Asp Thr		
246 850 855 860		
248 tgg aaa aat ata att gtc atg tat aac cca aat aag act tcc caa acc		2640
249 Trp Lys Asn Ile Ile Val Met Tyr Asn Pro Asn Lys Thr Ser Gln Thr		
250 865 870 875 880		
252 ctt aat cta cca agt gga gat tgg acc att gta gga ttg gga gat caa		2688
253 Leu Asn Leu Pro Ser Gly Asp Trp Thr Ile Val Gly Leu Gly Asp Gln		
254 885 890 895		
256 ata ggt gag aaa tca tta ggg cat gta atg ggt aat gtt caa gta ccg		2736
257 Ile Gly Glu Lys Ser Leu Gly His Val Met Gly Asn Val Gln Val Pro		

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258	900	905	910	
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267	<212> TYPE: PRT			
268	<213> ORGANISM: Bacillus acidopullulyticus			
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277	20	25	30	
280	Gly Asn Gly Ala Ala Tyr Gln Phe Thr Gly Thr Asn Asp Asp Phe Gly			
281	35	40	45	
284	Ala Val Ala Asp Thr Gln Val Pro Gly Asp Asn Thr Gln Val Gly Leu			
285	50	55	60	
288	Ile Val Arg Lys Asn Asp Trp Ser Glu Lys Asn Thr Pro Asn Asp Leu			
289	65	70	75	80
292	His Ile Asp Leu Ala Lys Gly His Glu Val Trp Ile Val Gln Gly Asp			
293	85	90	95	
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301	115	120	125	
304	Leu Ser Met Pro Met Thr Leu Ala Asp Ala Ala Ser Gly Phe Thr Val			
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308	Ile Asp Lys Thr Thr Gly Glu Lys Ile Pro Val Thr Ser Ala Val Ser			
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312	Ala Asn Pro Val Thr Ala Val Leu Val Gly Asp Leu Gln Gln Ala Leu			
313	165	170	175	
316	Gly Ala Ala Asn Asn Trp Ser Pro Asp Asp Asp His Thr Leu Leu Lys			
317	180	185	190	
320	Lys Ile Asn Pro Asn Leu Tyr Gln Leu Ser Gly Thr Leu Pro Ala Gly			
321	195	200	205	
324	Thr Tyr Gln Tyr Lys Ile Ala Leu Asp His Ser Trp Asn Thr Ser Tyr			
325	210	215	220	
328	Pro Gly Asn Asn Val Ser Leu Thr Val Pro Gln Gly Glu Lys Val			
329	225	230	235	240
332	Thr Phe Thr Tyr Ile Pro Ser Thr Asn Gln Val Phe Asp Ser Val Asn			
333	245	250	255	
336	His Pro Asn Gln Ala Phe Pro Thr Ser Ser Ala Gly Val Gln Thr Asn			
337	260	265	270	
340	Leu Val Gln Leu Thr Leu Ala Ser Ala Pro Asp Val Thr His Asn Leu			
341	275	280	285	
344	Asp Val Ala Ala Asp Gly Tyr Lys Ala His Asn Ile Leu Pro Arg Asn			
345	290	295	300	
348	Val Leu Asn Leu Pro Arg Tyr Asp Tyr Ser Gly Asn Asp Leu Gly Asn			
349	305	310	315	320

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L:13 M:270 C: Current Application Number differs, Replaced Current Application Number  
L:14 M:271 C: Current Filing Date differs, Replaced Current Filing Date